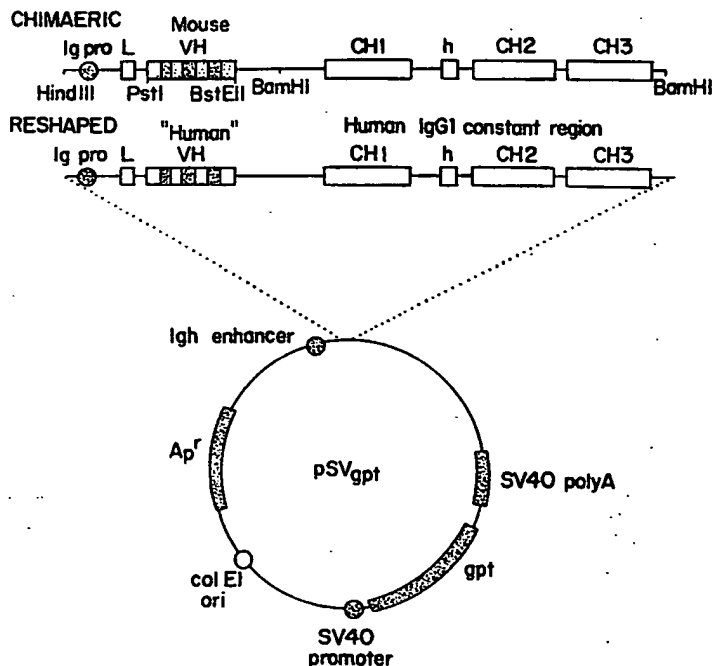




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12P 21/08, C07K 15/28	A1	(11) International Publication Number: WO 94/10332 (43) International Publication Date: 11 May 1994 (11.05.94)
<p>(21) International Application Number: PCT/US93/10384</p> <p>(22) International Filing Date: 4 November 1993 (04.11.93)</p> <p>(30) Priority data: 9223377.4 4 November 1992 (04.11.92) GB</p> <p>(71) Applicant (for all designated States except US): MEDAREX, INC. [US/US]; 22 Chambers Street, Princeton, NJ 08542 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only) : TEMPEST, Philip, R. [GB/GB]; 63 Brighton Place, Aberdeen AB1 6RT (GB). HARRIS, William, J. [GB/GB]; 3 Caesar Avenue, Carnoustie DD7 6DR (GB). CARR, Frank, J. [GB/GB]; Birchlea, The Holdings, Balmedie, Aberdeenshire AB23 8XU (GB).</p>	<p>(74) Agents: MANDRAGOURAS, Amy, E. et al.; Lahive & Cockfield, 60 State Street, Boston, MA 02109 (US).</p> <p>(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report.</i></p>	

(54) Title: **HUMANIZED ANTIBODIES TO Fc RECEPTORS FOR IMMUNOGLOBULIN G ON HUMAN MONONUCLEAR PHAGOCYTES**



(57) Abstract

Humanized antibodies are described which are specific to an Fc receptor (FcR). The humanized antibodies have at least a portion of a complementarity determining region (CDR) derived from a non-human antibody, e.g. murine, with the remaining portions being human in origin. The use of humanized antibodies rather than murine antibodies in human therapy should alleviate some of the problems associated with the use of some murine monoclonal antibodies because only the substituted CDRs will be foreign to a human's immune system. The humanized antibodies can be used in the same manner as their murine counterparts.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

HUMANIZED ANTIBODIES TO Fc RECEPTORS FOR IMMUNOGLOBULIN G ON HUMAN MONONUCLEAR PHAGOCYTES

5 Background

Human Fcγ receptors (FcγR) (reviewed in Fanger, M.W., et al. (1989) Immunology Today 10:92-99), of which there are three structurally and functionally distinct types (i.e., FcγRI, FcγRII and FcγRIII), are well-characterized cell surface glycoproteins that mediate phagocytosis or antibody-dependent cell cytotoxicity (ADCC) of immunoglobulin G
10 (IgG) opsonized targets. Antibodies have been made which are directed towards FcγR for various purposes, e.g., targeting of immunotoxins to a particular target cell type, or radioimaging a particular target cell type. The antibodies typically have been murine antibodies.

Murine monoclonal antibodies are sometimes desirable for human therapeutic
15 applications because the antibodies can be purified in large quantities and are free of contamination by human pathogens such as the hepatitis or human immunodeficiency virus. Murine monoclonal antibodies have been used in some human therapies, however, results have not always been desirable due to the development of an immune response to the "foreign" murine proteins. The immune response has been termed a human anti-mouse
20 antibody or HAMA response (Schroff, R., et al. (1985), Cancer Res., 45, 879-885) and is a condition which causes serum sickness in humans and results in rapid clearance of the murine antibodies from an individual's circulation. The immune response in humans has been shown to be against both the variable and the constant regions of the murine immunoglobulin.

Recombinant DNA technology has provided the ability to alter antibodies by
25 substituting specific immunoglobulin regions from one species with immunoglobulin regions from another species. Neuberger et al. (Patent Cooperation Treaty Patent Application No. PCT/GB85/00392) describes a process whereby the complementary heavy and light chain variable domains of an Ig molecule from one species may be combined with the complementary heavy and light chain Ig constant domains from another species. This
30 process may be used to substitute the murine constant region domains to create a "chimeric" antibody which may be used for human therapy. A chimeric antibody produced as described by Neuberger et al. would have the advantage of having the human Fc region for efficient stimulation of antibody mediated effector functions, such as complement fixation, but would still have the potential to elicit an immune response in humans against the murine ("foreign")
35 variable regions.

Winter (British Patent Application Number GB2188538A) describes a process for altering antibodies by substituting the complementarity determining regions (CDRs) with those from another species. This process may be used to substitute the CDRs from the murine variable region domains of a monoclonal antibody with desirable binding properties

(for instance to a human pathogen) into human heavy and light chain Ig variable region domains. These altered Ig variable regions may then be combined with human Ig constant regions to create antibodies which are totally human in composition except for the substituted murine CDRs. The "reshaped" or "humanized" antibodies described by Winter elicit a considerably reduced immune response in humans compared to chimeric antibodies because of the considerably less murine components. Further, the half life of the altered antibodies in circulation should approach that of natural human antibodies. However, as stated by Winter, merely replacing the CDRs with complementary CDRs from another antibody which is specific for an antigen such as a viral or bacterial protein, does not always result in an altered antibody which retains the desired binding capacity. In practice, some amino acids in the framework of the antibody variable region interact with the amino acid residues that make up the CDRs so that amino acid substitutions into the human Ig variable regions are likely to be required to restore antigen binding.

15 Summary of the Invention

The present invention pertains to humanized antibodies specific to an Fc receptor (FcR). The humanized antibodies have at least a portion of a complementarity determining region (CDR) derived from a non-human antibody, e.g., murine, with the remaining portions being human in origin. The use of humanized antibodies rather than murine antibodies in human therapy should alleviate some of the problems associated with the use of some murine monoclonal antibodies because only the substituted CDRs will be foreign to a human host's immune system.

The present invention further pertains to the use of humanized antibodies specific to an FcR as components in heteroantibodies, bifunctional antibodies, or immunotoxins. The humanized antibody specific to an FcR may be used in the same manner and for the same purpose as its corresponding murine counterpart. For example, the humanized anti-Fc receptor antibody of this invention can be used to treat cancer, allergies, and infectious and autoimmune diseases. Diagnostic applications of the antibodies include their use in assays for FcRI levels and assays for substances that influence FcR levels.

30

Brief Description of the Drawings

Figure 1 compares the amino acid sequences of murine 022 VH (SEQ ID NO:3) with the amino acid sequences of humanized NEWM-based VH (022 NMVH) (SEQ ID NO:1) and humanized KOL-based VH (022 KLVH) (SEQ ID NO:2). The CDRs are boxed. Murine residues retained in the human portion are indicated by the inverted black triangles.

Figure 2 compares the amino acid sequences of murine 022 VK (SEQ ID NO:28) with humanized REI-based VK (022 HuVK) (SEQ ID NO:4). The CDRs are boxed. Murine residues retained in the human portion are indicated by the inverted black triangles.

Figure 3 depicts the vector used for expression of the humanized or chimeric 022 heavy chain gene.

Figure 4 depicts the vector used for expression of the humanized or chimeric 022 kappa chain gene.

Figure 5 depicts the binding of the test antibodies in the enzyme linked immunoassay described in the example.

Detailed Description

The present invention pertains to a humanized antibody specific for an Fc receptor. The humanized antibody is made up of a human antibody having at least a portion of a complementarity determining region (CDR) derived from a non-human antibody. The portion is selected to provide specificity of the humanized antibody for a human Fc receptor. The humanized antibody has CDR's derived from a non-human antibody and the remaining portions of the antibody molecule are human.

The antibody may be a complete antibody molecule having full length heavy and light chains or any fragment thereof, e.g., Fab or (Fab')₂ fragment. The antibody further may be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct as described in Ladner et al. (U.S. Patent No. 4,946,778, issued August 7, 1990), the contents of which is expressly incorporated by reference.

The human antibody of the present invention may be any human antibody capable of retaining non-human CDRs. The preferred human antibody is derived from known proteins NEWM and KOL for heavy chain variable regions (VHs) and REI for Ig kappa chain, variable regions (VKs). These proteins are described in detail in the examples below.

"Complementarity determining region" (CDR) is an art recognized terminology and the technique used for locating the CDRs within the described sequences also is conventional.

The portion of the non-human CDR inserted into the human antibody is selected to be sufficient for allowing binding of the humanized antibody to the Fc receptor. A sufficient portion may be selected by inserting a portion of the CDR into the human antibody and testing the binding capacity of the created humanized antibody using the enzyme linked immunosorbent assay (ELISA) described in the examples below.

All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the

humanized antibody to the Fc receptor. The exemplified non-human CDR is derived from a murine antibody, particularly the CDR is derived from a monoclonal antibody (mab), mab 22. The mab 22 antibody is specific to the Fc receptor and further is described in U.S patent application Serial No. 07/151,450, filed on February 2, 1988, and in Fanger et al. (U.S. Patent
5 No. 4,954,617, issued September 4, 1988). The entire contents of the aforementioned pending application and issued patent are expressly incorporated by reference.

The CDRs are derived from a non-human antibody specific for a human Fc receptor. The CDRs can be derived from known Fc receptor antibodies such as those discussed in the Fanger et al. patent application and issued patent cited above (hereinafter
10 Fanger et al.). The CDR may be derived from an antibody which binds to the Fc receptor at a site which is not blocked by human immunoglobulin G. The antibody also may be specific for the high affinity Fc receptor for human immunoglobulin G. Examples of antibodies from which the non-human CDRs may be derived are mab 32, mab 22, mab 44, mab 62, mab 197 and anti-FcRI antibody 62. The humanized mab 22 antibody producing cell line has been
15 deposited at the American Type Culture Collection on November 4, 1992 under the designation HA022CL1 and has the accession no. CRL 11177.

The present invention also pertains to bifunctional antibodies or heteroantibodies having at least one humanized antigen binding region derived from a humanized anti-Fc receptor antibody and at least one antigen binding region specific for a
20 target epitope. The humanized antigen binding region may be derived from a humanized anti-Fc receptor antibody as described above. Bifunctional and heteroantibodies having an antibody portion specific for an Fc receptor are described in detail by Fanger et al.

It should be understood that the humanized antibodies of the present invention may be used in the same manner, e.g., as components of immunotoxins or heteroantibodies,
25 as their corresponding non-humanized counterparts described by Fanger et al. The humanized antibodies further share the same utilities as their non-humanized counterparts. All aspects of the teachings of the Fanger et al. application and patent are incorporated by reference.

The humanized antibody of the present invention may be made by any method
30 capable of replacing at least a portion of a CDR of a human antibody with a CDR derived from a non-human antibody. Winter describes a method which may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987), the contents of which is expressly incorporated by reference. The human CDRs may be replaced with non-human CDRs using oligonucleotide site-directed
35 mutagenesis as described in the examples below.

The humanized antibody of the present invention may be made as described in the brief explanation below. A detailed method for production is set forth in the examples. It should be understood that one of ordinary skill in the art may be able to substitute known

conventional techniques for those described below for the purpose of achieving the same result. The humanized antibodies of the present invention may be produced by the following process:

- 5 (a) constructing, by conventional techniques, an expression vector containing an operon with a DNA sequence encoding an antibody heavy chain in which the CDRs and such minimal portions of the variable domain framework region that are required to retain antibody binding specificity are derived from a non-human immunoglobulin, and the remaining parts of the antibody chain are derived from a human immunoglobulin, thereby producing the vector of the invention;
- 10 (b) constructing, by conventional techniques, an expression vector containing an operon with a DNA sequence encoding a complementary antibody light chain in which the CDRs and such minimal portions of the variable domain framework region that are required to retain donor antibody binding specificity are derived from a non-human immunoglobulin, and the remaining parts of the antibody chain are derived from a human immunoglobulin,
- 15 thereby producing the vector of the invention;
- (c) transfecting the expression vectors into a host cell by conventional techniques to produce the transfected host cell of the invention; and
- (d) culturing the transfected cell by conventional techniques to produce the altered antibody of the invention.

20 The host cell may be cotransfected with the two vectors of the invention, the first vector containing an operon encoding a light chain derived polypeptide and the second vector containing an operon encoding a heavy chain derived polypeptide. The two vectors contain different selectable markers, but otherwise, apart from the antibody heavy and light chain coding sequences, are preferably identical, to ensure, as far as possible, equal

25 expression of the heavy and light chain polypeptides. Alternatively, a single vector may be used, the vector including the sequences encoding both the light and the heavy chain polypeptides. The coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both.

The host cell used to express the altered antibody of the invention may be

30 either a bacterial cell such as Escherichia coli, or a eukaryotic cell. In particular a mammalian cell of a well defined type for this purpose, such as a myeloma cell or a Chinese hamster ovary cell may be used.

The general methods by which the vectors of the invention may be constructed, transfection methods required to produce the host cell of the invention and

35 culture methods required to produce the antibody of the invention from such host cells are all conventional techniques. Likewise, once produced, the humanized antibodies of the invention may be purified according to standard procedures of the art, including cross-flow

filtration, ammonium sulphate precipitation, affinity column chromatography, gel electrophoresis and the like.

It should be understood that the humanized antibodies of this invention perform in a manner which is the same or similar to that of the non-humanized versions of the same antibodies. It also is noted that the humanized antibodies of this invention may be used for the design and synthesis of either peptide or non-peptide compounds (mimetics) which would be useful for the same therapy as the antibody (Saragobi et al., Science 253:792-795 (1991)), the contents of which is expressly incorporated by reference.

The following examples are provided as a further illustration of the present invention and should in no way be construed as being limiting.

EXAMPLES

In the following examples all necessary restriction and modification enzymes, plasmids and other reagents and materials were obtained from commercial sources unless otherwise indicated.

In the following examples, unless otherwise indicated, all general recombinant DNA methodology was performed as described in "Molecular Cloning, A Laboratory Manual" (1982) Eds T. Maniatis et al., published by Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, the contents of which is expressly incorporated by reference.

In the following examples the following abbreviations were employed:

dCTP	deoxycytidine triphosphate
dATP	deoxyadenosine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
DTT	dithiothreitol
C	cytosine
A	adenine
G	guanine
T	thymine
PBS	phosphate buffered saline
PBST	phosphate buffered saline containing 0.05% Tween 20 (pH 7.5)

Example 1 - Production of Humanized Antibodies Specific for an Fc Receptor

The source of the donor CDRs used to prepare the humanized antibody was a murine monoclonal antibody, mab 22, which is specific for the Fc receptor. A mab 22

-7-

hybridoma cell line (022WCL-1) was established. Cytoplasmic RNA was prepared from the mab 22 cell line using the method described by Favoloro et al. (Methods in Enzymology 65, 718-749 (1980)), the contents of which is expressly incorporated by reference. The cDNA was synthesized using IgG1 and kappa constant region primers. The primer CG1FOR was used for the heavy chain variable (VH) region and the primer CK2FOR was used for the Ig kappa chain variable region (VK). The cDNA synthesis reactions mixtures consisted of 1 µg RNA, 0.5µM CG1FOR or CK2FOR, 250 µM each of dATP, dCTP, dGTP, and dTTP, 50 mM Tris HCl (pH 7.5), 75 mM KCl, 10 mM dithiothreitol, 3 mM MgCl₂ and 20µ RNAGuard (sold by Pharmacia, Milton Keynes, U.K.) in a total volume of 50 µl. The samples were heated at 72°C for two minutes and slowly cooled to 37°C. Murine moloney leukemia virus reverse transcriptase (100 µl - sold by Life Technologies, Paisley, U.K.) was added to the samples and the transcriptase containing samples were incubated at 42°C for sixty minutes.

VH and VK cDNAs were then amplified using the polymerase chain reaction (PCR) as described by Saiki et al. (Science 239, 487-491 (1988)), the contents of which is expressly incorporated by reference. The primers used in the above steps were as follows:

CG1FOR (SEQ ID NO:5) 5' GGAAGCTTAGACAGATGGGGGTGTCGTTTTG 3'
 VH1FOR (SEQ ID NO:6) 5' TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG 3'
 VH1BACK (SEQ ID NO: 7) 5' AGGTSMARCTGCAGSAGTCWGG 3'
 SH1BACK (SEQ ID NO:8) 5' TGGAATTCATGGRATGGAGCTGGRTCTWTBHTCTT 3'
 SH2BACK (SEQ ID NO:9) 5' TGGAATTCATGRACCTCDGGYTCAACTKRRTTT 3'
 CK2FOR (SEQ ID NO:10) 5' GGAAGCTTGAAGATGGATACAGTTGGTGCAGC 3'
 VK1BACK (SEQ ID NO:11) 5' GACATTCAGCTGACCCAGTCTCCA 3'
 VK5BACK (SEQ ID NO:12) 5' TTGAATTCGGTGCCAGAKCWSAHATYGTKATG 3'
 VK6BACK (SEQ ID NO:13) 5' TTGAATTCGGTGCCAGAKCWSAHATYGTKCTC 3'
 VK7BACK (SEQ ID NO:14) 5' TTGAATTCGGAGCTGATGGGAACATTGTAATG 3'

Restriction sites incorporated in primers to facilitate cloning are underlined.

The PCR amplification of murine Ig DNA was conducted using the methodology described by Orlandi et al. (Proc. Natl. Acad. Sci USA 86, 3833-3838 (1989)), the contents of which is expressly incorporated by reference. The DNA/primer mixtures consisted of RNA/cDNA hybrid (10 µl) and 25pmol each of CG1FOR and SH1BACK or SH2BACK for PCR amplification of VH. The DNA/primer mixtures consisted of RNA/cDNA hybrid (10 µl) and 25pmol each of CK2FOR and VK1BACK, VK5BACK, VK6BACK, VK7BACK for PCR amplification of VK. dATP, dCTP, dGTP and dTTP (250

5 μ M each), 10mM Tris HCl (pH 8.3), 60mM KCl, 1.5mM MgCl₂, 0.01% (w/v) gelatin, 0.01% (v/v) Tween 20, 0.01% (v/v) NP40 and 2.5 μ Amplitaq (sold by Cetus, Beaconsfield, U.K.) were added to the samples in a final volume of 50 μ l. The samples were subjected to 25-30 thermal cycles of PCR at 94°C for thirty seconds, 55°C for thirty seconds, 72°C for one minute and a final cycle at 72°C for five minutes.

The amplified VH and VK DNAs were run on a low melting point agarose gel and purified by Elutip-d column chromatography (sold by Schleicher and Schuell, Anderman, Walton, U.K.) for cloning and sequencing. The purified VH DNAs were cut with Eco I or Pst I and Hind III and cloned into M13mp18 and mp19 (sold by Pharmacia, Milton Keynes, U.K.). The purified VK DNAs were cut with Pvu II or Eco I and Hind III and cloned into M13mp18 and mp19. For general cloning methodologies see Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)), the contents of which are expressly incorporated by reference. The resulting collection of clones were sequenced by the dideoxy method using T7 DNA polymerase (sold by Pharmacia, Milton Keynes, U.K.) as described by Sanger et al. (Proc. Natl. Acad. Sci. USA 74, 5463-5467, (1979)), the contents of which are expressly incorporated by reference.

From the sequences of the O22 VH and VR domains the CDR sequences were determined with reference to the database of Kabat et al. ("Sequences of Proteins of Immunological Interest" US Department of Health and Human Services, US Government Printing Office), the contents of which is expressly incorporated by reference, and utilizing computer assisted alignment with other VH and VK sequences.

Transfer of the murine O22 CDRs to human frameworks was achieved by oligonucleotide site-directed mutagenesis as described by Nakamye et al. (Nucleic Acids Res 14, 9679-9687 (1986)), the contents of which is expressly incorporated by reference. The primers used were as follows:

KL VHCDR1 (SEQ ID NO:15): 5' TGCCTGTCTCACCCAATACATGTAA
TTGTCACTGAAATGAAGCCAGACGMGGAGCGGACAG
30
KL VHCDR2 (SEQ ID NO:16): 5' TGTAATCTTCCCTTCACACTGTCTGGATAGTA
GGTGTAACCTACCACCATCACTAATGGTTGCAACCCACTCAGG
KL VHCDR3 (SEQ ID NO:17): 5' GGGGTCCCTTGGCCCCAGTAGTCCATAGC
35 CCCCTCGTACCTATAGTAGCCTCTTGCAAAAAATAGA
NM VHCDR1 (SEQ ID NO:18): 5' TGGCTGTCTCACCCAATACATGTAATTGT
CGCTGAAATGAAGCCAGACACGGTGCAGGTCAGGCTCA

NMVHCDR2 (SEQ ID NO:19): 5' TTGCTGGTGTCTCTCAGCATTGTCACTCTC
 CCCTTCACACTGTCTGGATAGTAGGTGTAACCTACCACCA
 TCACTAATGGTTCCAATCCACTCAA

5

NMVHCDR3 (SEQ ID NO:20): 5' AGACGGTGACCAAGGACCCTTGGCCCCAG
 TAGTCCATAGCCCCCTCGTACCTATAGTAGCCTCTTGCACAATAATAG

HuVKCDR1 (SEQ ID NO:21): 5' CTTCTGCTGGTACCAGGCCAAGTAGTTCTTC
 10 TGATTTGAACTGTATAAAACACTTTGACTGGACTTACAGGTGATGGTCAC

HuVKCDR2 (SEQ ID NO:22): 5' GCTTGGCACACCAGATTCCCTAGTGGATG
 CCCAGTAGATCAGCAG

15 HuVKCDR3 (SEQ ID NO:23): 5' CCTTGGCCGAACGTCCACGAGGAGAGGTAT
 TGATGGCAGTAGTAGGTGG

20 The primer for NMVHCDR1 was extended to include a change of NEWM residues Ser 27
 Thr 28 to Phe 27 Ile 28. The primer for NMVHCDR2 was extended to include a change of
 NEWM residue Val 71 to Arg 71.

The DNA templates used for mutagenesis of VHs comprised human
 framework regions from the crystallographically solved protein NEW described by Saul et al.
 (J. Biol. Chem. 53, 585-597 (1978)) or KOL described by Schmidt et al. (Z. Physical Chem.
 25 364, 713-747 (1983)). The DNA templates used for mutagenesis of VKs comprised human
 framework regions from the crystallographically solved protein REI described by Epp et al.
 (Eur. J. Biochem. 45, 513-524 (1974)). The contents of each of the forementioned references
 are expressly incorporated by reference.

M13 based templated M13VHPCR1 (for NEWMVH), M13VHPCR2 (for
 30 KOLVH) and M13VKPCR2 (for REIVK) comprising human frameworks with irrelevant
 CDRs were prepared as described by Riechmann et al. (Nature 332, 323-327 (1988)), the
 contents of which are expressly incorporated by reference. Oligonucleotide site-directed
 mutagenesis was carried out using the following protocol. A 5-fold molar excess of each
 phosphorylated mutagenic oligonucleotide was added along with the universal M13
 35 sequencing primer (5'- GTAAAACGACGGCCAGT) (SEQ ID NO:24). All of the primers
 were annealed in 20ul 0.1M TrisHCl (pH8.0) and 10mM MgCl₂ by heating to 70-85°C for
 two minutes and slowly cooling to room temperature. 10 mM DTT, 1 mM ATP, 40 µM each
 of dATP, dCTP, dGTP and dTTP, 2.5µ T7 DNA polymerase (sold by United States

Biochemicals) and 0.5 μ T4DNA ligase (sold by Life Technologies, Paisley, U.K.) was added to the annealed DNA in a reaction volume of 30 μ l and incubated at 22° - 37°C for one to two hours. The newly extended and ligated strand was preferentially amplified over the parental strand in a thermostable DNA polymerase directed reaction using the M13 reverse sequencing primer (5' AACAGCTATGACCATG) (SEQ ID NO:25). The reverse sequencing primer is not complementary to the parental strand. The reaction mixture of 50 μ l contained 1 μ l extension/ligation product, 25 pmol M13 reverse sequencing primer, 250 μ M each of dATP, dCTP, dGTP and dTTP, 1 μ Vent DNA polymerase (sold by New England Biolabs, Bishop's Stortford, U.K.) or 2.5 μ Amplitaq (sold by Cetus, Beaconsfield, U.K.) in the appropriate buffer supplied by the enzyme manufacturer and was subjected to thirty thermal cycles of 94°C, 30s, 55°C, 30s, 75° or 72°C, 90s; ending with 5 min at 72°C. A 4 μ l aliquot of this sample was then amplified by PCR using both M13 universal and reverse sequencing primers in a reaction mixture of 50 μ l containing 25 pmol of each primer, 250 μ M each of dATP, dCTP, dGTP and dTTP, 2.5 μ Amplitaq (Cetus) in the buffer supplied by the enzyme manufacturer. Amplified DNAs were digested with HindIII and BamHI and cloned into M13mp19 and sequenced.

Mutagenesis of M13VHPCR2 KOL VH residue Leu71 to Arg71 was by the overlap/extension PCR method of Ho *et al.* (*Gene*, 77, 51-55 (1989)), the contents of which is expressly incorporated by reference. The overlapping oligonucleotides used were 5' - TTTACAATATCGAGACAACAGCAA (SEQ ID NO:26) and 5' - TTGCTGTTGTCTCTCGATTGTAAA (SEQ ID NO:27).

The amino acid sequences of the humanized antibodies were compared to the known murine antibodies as shown in Figures 1 and 2. The CDR replaced VH and VK genes were cloned into expression vectors pSVgpt and pSVhyg as shown in Figures 3 and 4 as described by Orlandi *et al.* (cited supra). The CDR replaced NEWMVH and KOLVH genes together with the Ig heavy chain promoter, appropriate splice sites and signal peptide sequences were excised from M13 by digestion with HindIII and BamHI and cloned into the pSVgpt expression vector containing the murine Ig heavy chain enhancer, the gpt gene for selection in mammalian cells and genes for replication and selection in *E. coli*. The plasmid also contains a human IgGI constant region as described by Takahashi *et al.* (*Cell* 29, 671-675 (1982)). The construction of the kappa chain expression vector was essentially the same except that the gpt gene was replaced by the hygromycin resistance gene and contains a human kappa constant region (Hieter *et al.*, *Cell* 22, 197-207 (1980)). The contents of each of the forementioned references are expressly incorporated by reference.

Approximately 5 μ g of each heavy chain expression vector and 10 μ g of the kappa chain expression vector were digested with PvuI. The DNAs were mixed together, ethanol precipitated and dissolved in 25 μ l water. Approximately 5-10 x 10⁶ NSO cells (from European Collection of Animal Cell Cultures, Porton Down, U.K.) were grown to semi-

confluency in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal calf serum (Myoclonal plus, Gibco, Paisley, Scotland), harvested by centrifugation and resuspended in 0.5ml DMEM together with the digested DNA in a cuvette. After five minutes in ice, the cells were given a single pulse of 170V at 960 μ F (Gene-Pulser, Bio-Rad, Richmond, CA) and left in ice for a further twenty minutes. The cells were then put into 20ml DMEM + supplemented with 10% FCS and allowed to recover for twenty-four to forty-eight hours. After this time, the cells were distributed into a 24-well plate and selective medium was applied (DMEM, 10% FCS, 0.8 μ g/ml mycophenolic acid and 250 μ g/ml xanthine). After three to four days, the medium and dead cells were removed and replaced with fresh selective medium. Transfected clones were visible with the naked eye ten days later.

The presence of human antibody in the medium of wells containing gpt+ transfectants was measured using conventional enzyme linked immunosorbent assay (ELISA) techniques. Wells of a microtitre plate (Immolon, Dynatech, Chantilly, VA) were coated with 100ng goat anti-human IgG antibodies (SeraLab, Crawley Down, U.K.) in 100 μ l 50mM carbonate buffer pH9.6. After washing with PBST (Phosphate buffered saline pH 7.2 containing 0.05% Tween 20) culture medium in 100 μ l PBST (5-50 μ l) was added to each well for one hour at 37°C. The wells were then emptied, washed with PBST and 100 μ l of 1:1000 dilution peroxidase conjugated goat anti-human kappa constant region antibodies (SeraLab, Crawley Down, U.K.) were added for one hour at 37°C. The wells were emptied, washed with PBST and 100 μ l OPD substrate buffer (400 μ g/ml Q-phenylenediamine in 24mM citrate/42mM sodium phosphate pH 5. and 0.0003% (v/v) H₂O₂) was added. The reaction was stopped after a few minutes by the addition of 12.5% H₂SO₄ (25 μ l) and the absorbance at 492nm was measured.

The antibody secreting cells were expanded and antibody was purified from the culture medium by protein A affinity chromatography as described by Harlow and Lane (Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), the contents of which is expressly incorporated by reference.

The binding of the antibodies to antigen was measured by ELISA. Wells of a microtitre plate (Immulon 1, Dynatech, Chantilly, VA) were coated with 200ng goat anti-human IgM antibodies (Sera-lab, Crawley Down, U.K.) in 100 μ l 50mM carbonate buffer pH 9.6 at 37°C for at least one hour. Wells were emptied and washed once with PBST and blocked with 1% BSA in PBS at room temperature for thirty minutes. The wells were emptied and washed with PBST and Cos supernatant containing FcRI/IgM fusion protein was added and incubated for one hour at room temperature. Wells were then emptied and washed three times with PBST and test antibodies diluted in 1% BSA/PBS were added and incubated for one hour at room temperature. In addition, each well contained 2 μ g human IgG1, lambda antibody (Sigma, Poole, U.K.) The wells were then emptied, washed three times with PBST and 40ng peroxidase goat anti-human kappa constant region antibodies (Sera-Lab, Crawley

Down, U.K.) in 100µl 1% BSA/PBS added to each well. After incubation for one hour at room temperature, the wells were emptied, washed three time with PBST and 10µl HPD substrate buffer was added. The reaction was stopped by the addition of 25µl of 12.5% H₂SO₄ to each well. The absorbance at 492nm was measured and is depicted in Figure 5.

- 5 The test antibodies were the antibody containing irrelevant CDRs (AA), the fully humanized KOL/REI based antibody (KL VHR/HuVK), the mix and match derivatives of the humanized antibody (KL VHR/MuVK and MuVH/HuVK), the humanized NEWM/REI based antibody (NMVK/HuVK) and the chimeric antibody (MuVH/MuVK).

10 EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

-13-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

- (A) NAME: MEDAREX, INC.
- (B) STREET: 22 Chambers Street
- (C) CITY: Princeton
- 10 (D) STATE: New Jersey
- (E) COUNTRY: USA
- (F) POSTAL CODE (ZIP): 08542
- (G) TELEPHONE: (609) 921-7121
- (H) TELEFAX: (609) 921-7450

15

- (ii) TITLE OF INVENTION: HUMANIZED ANTIBODIES TO Fc RECEPTORS FOR
IMMUNOBLOBULIN G ON HUMAN MONONUCLEAR PHAGOCYTES

- (iii) NUMBER OF SEQUENCES: 28

20

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- 25 (D) SOFTWARE: ASCII text

25

(v) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- 30 (C) CLASSIFICATION:

30

(vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: GB92 23377.4
- (B) FILING DATE: 04-NOV-1992
- 35 (C) CLASSIFICATION:

35

(vii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Mandragouras, Amy E.
- (B) REGISTRATION NUMBER: 36.207
- 40 (C) REFERENCE/DOCKET NUMBER: MXI-013PC

40

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (617) 227-7400
- (B) TELEFAX: (617) 227-5941

45

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 120 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: peptide

55

-14-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

5 Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
 1 5 10 15
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ile Phe Ser Asp Asn
 20 25 30
 10 Tyr Met Tyr Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
 35 40 45
 Gly Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
 50 55 60
 15 Lys Gly Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
 65 70 75 80
 Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 20 Ala Arg Gly Tyr Tyr Arg Tyr Glu Gly Ala Met Asp Tyr Trp Gly Gln
 100 105 110
 25 Gly Ser Leu Val Thr Val Ser Ser
 115 120

(2) INFORMATION FOR SEQ ID NO: 2:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 120 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 35 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

40 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 45 Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe Ser Asp Asn
 20 25 30
 Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 50 Ala Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
 65 70 75 80
 55 Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
 85 90 95

-15-

Ala Arg Gly Tyr Tyr Arg Tyr Glu Gly Ala Met Asp Tyr Trp Gly Gln
 100 105 110

5 Gly Thr Pro Val Thr Val Ser Ser
 115 120

(2) INFORMATION FOR SEQ ID NO: 3:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 120 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

20 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 25 Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Phe Ile Phe Ser Asp Asn
 20 25 30
 Tyr Met Tyr Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
 35 40 45
 30 Ala Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Asn Leu Tyr
 65 70 75 80
 35 Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Ile Tyr Tyr Cys
 85 90 95
 40 Ala Arg Gly Tyr Tyr Arg Tyr Glu Gly Ala Met Asp Tyr Trp Gly Gln
 100 105 110
 Gly Thr Ser Val Thr Val Ser Ser
 115 120

45

(2) INFORMATION FOR SEQ ID NO: 4:

50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 112 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

55

-16-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

5 Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
 20 25 30
 10 Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
 35 40 45
 Ala Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
 50 55 60
 15 Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr
 65 70 75 80
 Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys His Gln
 85 90 95
 20 Tyr Leu Ser Ser Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105 110

25 (2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 base pairs
 (B) TYPE: nucleic acid
 30 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

40 GGAAGCTTAG ACAGATGGGG GTGTCGTTTT G 31

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 34 amino acids
 45 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

55 Thr Gly Ala Gly Gly Ala Gly Ala Cys Gly Gly Thr Gly Ala Cys Cys
 1 5 10 15

-17-

Gly Thr Gly Gly Thr Cys Cys Cys Thr Thr Gly Gly Cys Cys Cys Cys
20 25 30

Ala Gly

5

(2) INFORMATION FOR SEQ ID NO: 7:

- 10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

20

AGGTSMARCT GCAGSAGTCW GG

22

(2) INFORMATION FOR SEQ ID NO: 8:

- 25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30

- (ii) MOLECULE TYPE: cDNA

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TGGAATTCAT GGRATGGAGC TGGRTCWTBH TCTT

34

(2) INFORMATION FOR SEQ ID NO: 9:

40

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45

- (ii) MOLECULE TYPE: cDNA

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TGGAATTCAT GRACITCDGG YTCAACTKRR TTT

33

55

-18-

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

15 GGAAGCTTGA AGATGGATAC AGTTGGTGCA GC

32

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

30

GACATTCAGC TGACCCAGTC TCCA

24

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

45

TTGAATTCGG TGCCAGAKCW SAHATYGTKA TG

32

(2) INFORMATION FOR SEQ ID NO: 13:

50

(i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

-19-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TTGAATTCGG TGGCAGAKCW SAHATYGTTC TC

32

5 (2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TTGAATTCGG AGCTGATGGG AACATTGTAA TG

32

20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 61 base pairs

(B) TYPE: nucleic acid

25 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

35 TGCCTGTCTC ACCCAATACA TGTAATTGTC ACTGAAATGA AGCCAGACGM GGAGCGGACA

60

G

61

40 (2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 75 base pairs

(B) TYPE: nucleic acid

45 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TGTAATCTT CCCTTCACAC TGTCTGGATA GTAGGTGTAA CTACCACCAT CACTAATGGT

60

55 TGCAACCCAC TCAGG

75

-20-

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

15 GGGGTCCCTT GGCCCCAGTA GTCCATAGCC CCCTCGTACC TATAGTAGCC TCTTGCACAA 60
AAATAGA 67

(2) INFORMATION FOR SEQ ID NO: 18:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TGGCTGTCTC ACCCAATACA TGTAATTGTC GCTGAAAATG AAGCCAGACA CGGTGCAGGT 60
35 CAGGCTCA 68

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 94 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

50 TTGCTGGTGT CTCTCAGCAT TGTCACCTCTC CCCTTCACAC TGTCTGGATA GTAGGTGTAA 60
CTACCACCAT CACTAATGGT TCCAATCCAC TCAA 94

55

-21-

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

15 AGACGGTGAC CAAGGACCCT TGGCCCCAGT AGTCCATAGC CCCCTCGTAC CTATAGTAGC 60
CTCTTGACACA ATAATAG 77

20 (2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

35 CTTCTGCTGG TACCAGGCCA AGTAGTTCTT CTGATTTGAA CTGTATAAAA CACTTTGACT 60
GGACTTACAG GTGATGGTCA C 81

40 (2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

55 GCTTGGCACA CCAGATTCCC TAGTGGATGC CCAGTAGATC AGCAG 45

-22-

(2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CCTTGGCCGA ACGTCCACGA GGAGAGGTAT TGATGGCAGT AGTAGGTGG

49

(2) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GTAAACGAC GGCCAGT

17

(2) INFORMATION FOR SEQ ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

AACAGCTATG ACCATG

16

(2) INFORMATION FOR SEQ ID NO: 26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

-23-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

TTTACAATAT CGAGACAACA GCAA

24

5

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

10

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

20

TTGCTGTTGT CTCTCGATTG TAAA

24

(2) INFORMATION FOR SEQ ID NO: 28:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 112 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: peptide

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Asn Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
 1 5 10 15

40

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
 20 25 30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
 35 40 45

45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
 50 55 60

50

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys His Gln
 85 90 95

55

Tyr Leu Ser Ser Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

CLAIMS

1. A humanized antibody specific to an Fc receptor comprising:
a human antibody having at least a portion of a complementarity determining
5 region derived from a non-human antibody, the portion being selected to provide specificity
of the humanized antibody for a human Fc receptor.
2. The antibody of claim 1 wherein the portion is selected to provide specificity
to a human Fc receptor such that the humanized antibody formed binds to the Fc receptor at a
10 site which is not blocked by human immunoglobulin G.
3. The antibody of claim 1 wherein the portion is selected to provide specificity
of the humanized antibody for the high affinity Fc receptor for human immunoglobulin G.
- 15 4. The antibody of claim 1 wherein the portion of a complementarity determining
region is derived from a murine antibody.
5. The antibody of claim 1 wherein the portion of a complementarity determining
region is derived from a monoclonal antibody selected from the group consisting of mab 32,
20 mab 22, mab 44, mab 62, mab 197, and anti-FcRI antibody 62.
6. The antibody of claim 5 wherein the monoclonal antibody is mab 22.
7. The antibody of claim 1 wherein at least a portion of all of the
25 complementarity determining regions of the human antibody are derived from a non-human
antibody.
8. The antibody of claim 7 wherein the entire portion of all of the
complementarity determining regions of the human antibody are derived from a non-human
30 antibody.
9. The antibody of claim 7 wherein the non-human antibody is mab 22.
10. The antibody of claim 8 wherein the non-human antibody is mab 22.
- 35 11. The antibody of claim 1 wherein the human antibody is derived from proteins
selected from the group consisting of NEW, KOL, REI, and combinations thereof.

12. A bifunctional antibody or heteroantibody, comprising:
at least one humanized antigen binding region derived from a humanized anti-Fc receptor antibody, and
at least one antigen binding region specific for a target epitope.
- 5 13. The antibody of claim 12 wherein the humanized antigen binding region is derived from a humanized anti-Fc receptor antibody selected such that the binding of the humanized antibody to the human Fc receptor is not blocked by human immunoglobulin G.
- 10 14. The antibody of claim 13 wherein the humanized anti-Fc receptor antibody is specific for the high affinity Fc receptor for human immunoglobulin.
- 15 15. The antibody of claim 12 wherein the humanized antigen binding region has at least a portion of a complementarity determining region derived from a non-human antibody.
- 16 16. The antibody of claim 15 wherein the portion of a complementarity determining region is derived from a murine antibody.
- 20 17. The antibody of claim 15 wherein the portion of a complementarity determining region is derived from a monoclonal antibody selected from the group consisting of mab 32, mab 22, mab 44, mab 62, mab 197, and anti-FcRI antibody 62.
18. The antibody of claim 17 wherein the monoclonal antibody is mab 22.
- 25 19. The antibody of claim 15 wherein at least a portion of the all the complementarity determining regions of the human antibody are derived from a non-human antibody.
- 30 20. The antibody of claim 19 wherein the entire portion of all of the complementarity determining regions of the human antibody are derived from a non-human antibody.
21. The antibody of claim 19 wherein the non-human antibody is mab 22.
- 35 22. The antibody of claim 20 wherein the non-human antibody is mab 22.
23. The antibody of claim 12 wherein the target epitope is that of a cancer cell.

24. The antibody of claim 12 wherein the target epitope is that of an infectious agent.
25. The antibody of claim 12 wherein the target epitope is that of an antibody-producing cell.
- 5

1 / 6

	10V	20V	30V	40V	50V
022NMVH	QVQLQESGPGLVVRPSQTLSTCTVSGFIFSDNYMYWVRQPPGRGLEWIGTI				
	:VQL ESG GLV:P: :L.L:C..SGFIFSDNYMYWVRQ.P.: LEW::TI				
022MUVH	EVQLVESGGGLVKPGGSLRLSCVASGFI FSDNYMYWVRQTPEKRLIEWVATI				
	10^	20^	30^	40^	50^
	60V	70V	80V	90V	100V
022NMVH	SDGGSYTYYPDSVKGRVTMLRDTSKNQFSLRLSSVTAADTAVYVCARGYYR				
	SDGGSYTYYPDSVKGR T: RD.:KN:: L::SS:::DTA:YYCARGYYR				
022MUVH	SDGGSYTYYPDSVKGRFTISRDNAKNNLYLQMSSLKSEDTAIYYCARGYYR				
	60^	70^	80^	90^	100^
	110V	120V			
022NMVH	YEGAMDYWGQGS�TVSS				
	YEGAMDYWGQG: VTVSS				
022MUVH	YEGAMDYWGQGS�TVSS				
	110^	120^			

FIG. 1A

SUBSTITUTE SHEET (RULE 26)

022KLVH	10v	20v	30v	40v	50v
	EVQLVESGGGVQPGRSLRLSCSSSGFIFSDNYMYWVRQAPGKGLEWVATI				
	EVQLVESGGG:V:PG SLRLSC :SGFIFSDNYMYWVRQ:P.K LEWVATI				
022MUVH	10^	20^	30^	40^	50^
	EVQLVESGGGLVKPGSLRLSCVASGFIFSDNYMYWVRQTPEKRLEWVATI				
	60v	70v	80v	90v	100v
022KLVH	SDGGSYTYYPDSVKGRFTISRDN SKNTLFLQMDSL RPEDTGVYFCARGYYR				
	SDGGSYTYYPDSVKGRFTISRDN:KN.L1.QM.SLLEDTL1YLCARGYYR				
022MUVH	60^	70^	80^	90^	100^
	SDGGSYTYYPDSVKGRFTISRDN AKNNLYLQMSSSLKSEDTAIYYCARGYYR				
	110v	120v			
022KLVH	YEGAMDYWGQGTPTVTVSS				
	YEGAMDYWGQGT:VTVSS				
022MUVH	110^	120^			
	YEGAMDYWGQGTSTVTVSS				

FIG.1B

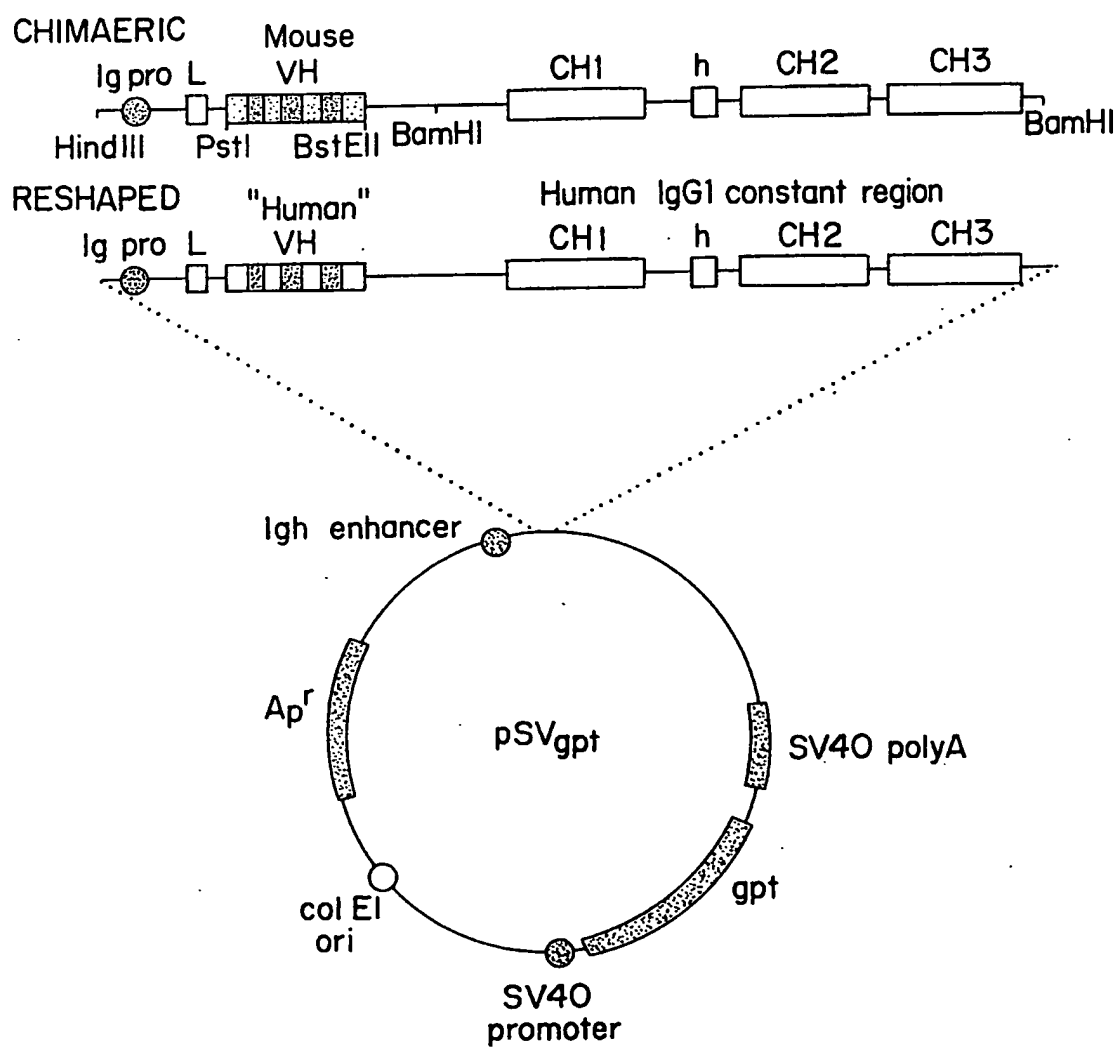
3 / 6

	10V	20V	30V	40V	50V
022MUVK	NIVMTQSPSSLAVSAGEKVTMSCKSSQSVLYSSNQKNYLAWYQQKPGQSPK				
	:I :TQSPSSL: .S.G.:VT::CKSSQSVLYSSNQKNYLAWYQQKPG::PK				
022HUVK	DIQLTQSPSSLASVGDRTITCKSSQSVLYSSNQKNYLAWYQQKPGKAPK				
	10 [^]	20 [^]	30 [^]	40 [^]	50 [^]
	60V	70V	80V	90V	100V
022MUVK	LLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSWT				
	LLIYWASTRESGVP.RF:GSGSGTDFTLTISS:Q:ED:A.YYCHQYLSSWT				
022HUVK	LLIYWASTRESGVPDRFSGSGSGTDFTFISSLQPEDIAITYYCHQYLSSWT				
	60 [^]	70 [^]	80 [^]	90 [^]	100 [^]
	110V				
022MUVK	FGGGTKLEIK				
	FG GTK:EIK				
022HUVK	FGQGTKVEIK				
	110 [^]				

FIG. 2

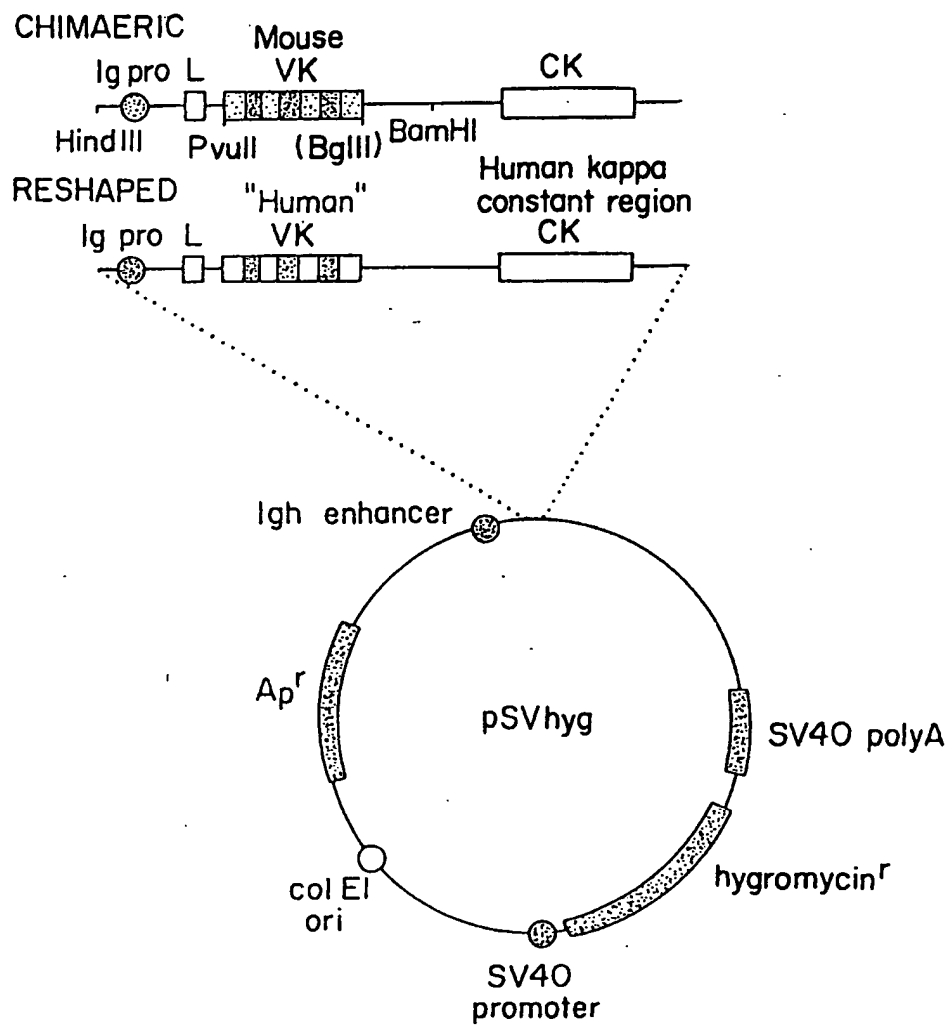
4 / 6

FIG. 3



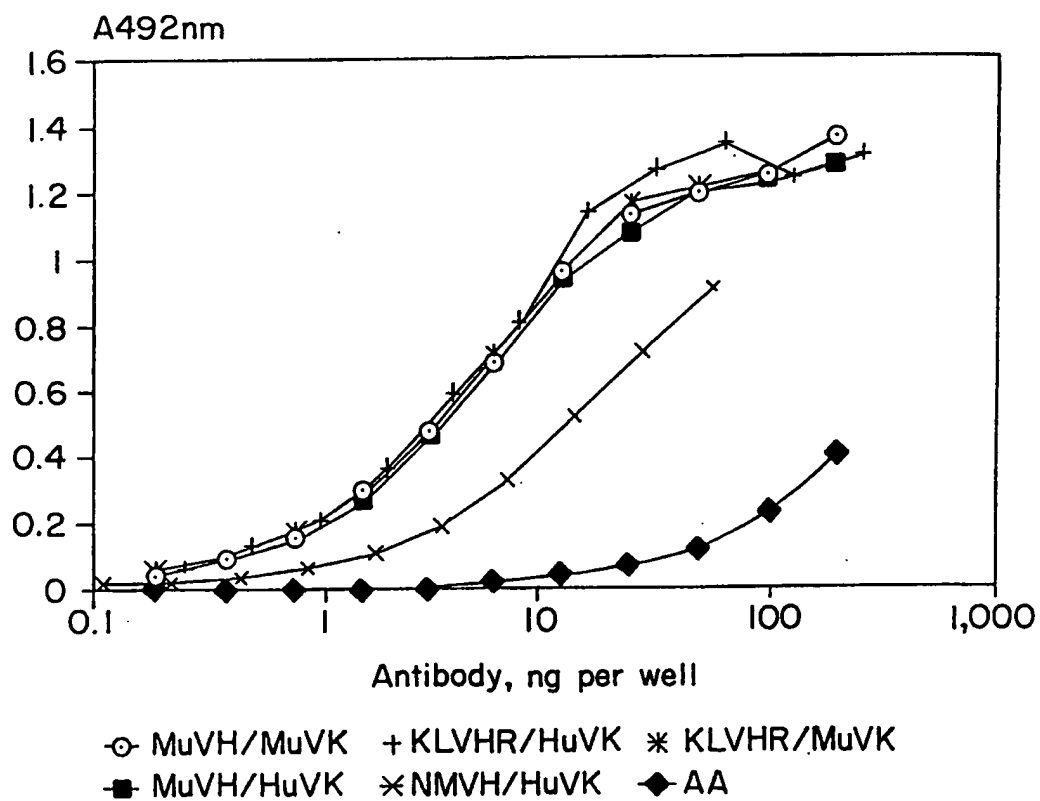
5 / 6

FIG. 4



6 / 6

FIG. 5



INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 93/10384

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12P21/08 C07K15/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 340 002 (K. TSUJI) 2 November 1989 see claims see example 8 see table 4 ---	1,2
Y	EP,A,0 255 249 (TRUSTEES OF DARTMOUTH COLLEGE) 3 February 1988 cited in the application see the whole document ---	1-25
Y	NATURE vol. 332 , 24 March 1988 , LONDON, GB pages 323 - 327 L. RIECHMANN ET AL. 'Reshaping human antibodies for therapy.' cited in the application see the whole document ---	1-25

-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

2 February 1994

Date of mailing of the international search report

21. 02. 94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Nooi, F

INTERNATIONAL SEARCH REPORT

Intern. Pat. Application No
PCT/US 93/10384

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>GENE vol. 101, no. 2 , 1991 , AMSTERDAM, THE NETHERLANDS pages 297 - 302 A. LEWIS ET AL. 'Immunoglobulin complementarity-determining region grafting by recombinant polymerase chain reaction to generate humanised monoclonal antibodies.' see the whole document -----</p>	1

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 93/10384

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0340002	02-11-89	JP-A- 2049597	19-02-90

EP-A-0255249	03-02-88	US-A- 4954617	04-09-90
		AU-B- 605771	24-01-91
		AU-A- 7527187	14-01-88
		CA-A- 1319899	06-07-93
		WO-A- 8800052	14-01-88
		JP-T- 1500195	26-01-89
